SCIENCE REVIEW OF A PRECONSTRUCTION CAGED BIVALVE STUDY FOR THE MARINE BASELINE MONITORING PROGRAM AT THE SYDNEY HARBOUR, NOVA SCOTIA

Context

At the end of December 2009, the Environmental Assessment and Major Projects (EAMP) division of the Oceans, Habitat, and Species at Risk Branch in the Maritimes Region requested that DFO Maritimes Science undertake a review of a document entitled "Marine Baseline Monitoring Program for Preconstruction Caged Bivalve Study." EAMP requested DFO Science advice on the report related to two issues:

i) Is the design of the environmental effects monitoring (EEM) program effective in determining any potential negative environment effects from the Sydney Tar Ponds and Coke Ovens Sites Remediation Project?
ii) Do the results of the EEM reflect the prediction of no significant adverse environmental effects in Sydney Harbour from the Sydney Tar Ponds and Coke Ovens Sites Remediation Project?

This information will be used to refine the EEM program and/or adjust mitigation measures to ensure environmental protection objectives are met. It was requested that a response be provided within a few weeks. Given the short timeframe for review, DFO's Science Special Response Process was used.

Background

On October 1, 2007, after a panel review under the Canadian Environmental Assessment Act (CEAA), the Government of Canada permitted the Sydney Tar Ponds and Coke Ovens Sites Remediation Project to proceed. Recommendation 19 of the Panel Report stated, "The Panel recommends that PWGSC [Public Works and Government Services Canada], in consultation with NRCan [Natural Resources Canada], DFO, Environment Canada, and the STPA [Sydney Tar Ponds Agency], design a long-term monitoring program to document improvements in the environmental quality of Sydney Harbour. DFO should assume the lead for long-term monitoring." As stated in the Government of Canada response to the Panel Report, DFO would not assume the lead but is a key player in the review of the EEM program.

In 2008 the Sydney Tar Ponds Agency initiated an environmental effects monitoring (EEM) program for Sydney Harbour with the overall objective of measuring the preconstruction (baseline) conditions for the Muggah Creek receiving waters. The objective of the present baseline study is to undertake preconstruction caged bivalve bioassays as a component of the marine EEM program.

Response

In general, the sampling program is well designed and the study report provides good baseline information for future monitoring. However, there are a number of improvements that could be made to the report.

Monitoring Locations

The terms of reference required that 9 monitoring sites be chosen: 3 near-field sites (Area 1); 3 mid-field sites (Area 2); 2 far field sites (Area 3), and 1 site near the Sydney River inflow (Area 4). While 9 sites were used for the study, the positioning of the cages was considered less than optimum in numerous areas. Within Area 1, sites are placed along the western shore the South Arm. Due to their location, sites in Area 1 may not be near field sites as intended. The sites should have been placed in Muggah Creek and a few meters with the Southern Arm. Within Area 2, there are 2 rather then 3 sites as indicated in the terms of reference. Site 5 should be incorporated as a mid-field site in Area 2 and a site should be placed on the south-eastern shore, north of the mouth of Muggah Creek. Sites within Area 3 are positioned in close proximity to one another and provide a restricted view of contaminant uptake by the mussels. Within Area 4, a site should have been placed on the eastern shore, rather than 2 on the western side of the Arm.

The location of construction and effluent discharge sites are not indicated on the site map (Figure 2.1) within the report. Likewise, the direction of water currents and circulation within Sydney Harbour are not indicated on a map or described in the text. These parameters play a role in the transport of effluents towards the caged mussels and may help interpret study results. It would be helpful if the rationale for site location was included in future reports.

Monitoring of Mussels

There are two different species of *Mytilus* in Nova Scotia waters. These two species are similar in appearance and often co-occur. *M. trossulus* has a lighter and longer shell than *M. edulus*, affecting the use of shell length as a covariate and measures of energy requirements for shell growth. A description of the species identification method(s) used to select mussels for the study would be helpful in future reports.

The use of caged mussels within Sydney Harbour was chosen as the monitoring approach for the study. Thus, population endpoints, such as the sex ratio and species composition (*M. edulis*, *M. trossulus* and triploids) of the natural population within the study area could not be pursued.

Although the study undertook contaminant analysis for both fall and spring samples, only 2 replicates per cage were analysed. The terms of reference indicated that three replicates (composites of 10 mussels each) were required for each station. Statistical analysis by station and season for the contaminants was inappropriate and only area-based analyses could be completed. Given the difference by station shown for the condition factor (CF), gonadosomatic index (GSI), and egg counts, consideration should be given to analyzing 3 samples per station for future monitoring.

It is important to note that the reproductive status of mussels will influence their weight. Any measure of reproductive status will vary seasonally with the development and release of the gametes. As gametes make up a variable portion of the body weight in mature mussels, condition factor will vary seasonally with the development of gametes. Unlike the data from November 2008, the May 2009 data is separated by sex (as mussels were ripe enough to sex at the time of year) and indicated the importance of seasonal and sex differences in condition factor analysis.

Statistical Analysis

There are more appropriate methods of statistical analysis that could have been used throughout this report. Most variables were log10 transformed to meet the assumptions of normality and homogeneity of variances. Log transforming data linearizes the cubic relationship; however, there are methods that would allow the analysis of original data as intended. An alternative approach that should be considered is the use of Generalised Linear Models (GLMs). GLMs do not require the assumptions of normality and homogeneity of variances and can, therefore, be used to analyse non-normal and non-homogenous data sets. These models do not require any transformation and have more power to detect differences between groups. Furthermore, during the discussion of condition factor (page 12), it is stated that there are concerns with the use of derived variables/ratio and condition factors were not subjected to statistical analysis for this reason. The same argument, however, is relevant for other variables. Analysis indicating if the transformation was successful should be presented in the report.

To decrease the number of statistical tests employed, the inclusion of sex as a factor in a single analysis should be considered. For example, condition factor was replaced with an ANCOVA (analysis of covariance) on log10(meat weight) with log10(length) as a covariate. Statistical methods that use the original data with sex as a factor in the analysis should be considered.

Egg abundance was analysed with an ANCOVA on log10(egg count) with log10(plug weight[1]) as a covariate. Egg counts have to be normalized to plug weight, and there should be detail in the report of the protocol used to ensure the same section is taken from all mussels. The counts could be normalized to a constant plug weight and the covariate removed.

The statistical analysis in Table 3.8 and 3.9 is considered a repeat of the condition factor test but using mantle weights. As reported on Page 9, shell and tissue growth are not directly linked and can occur at different times of the year. Adding length as a covariate adds more variability to the data that is not necessary. Relating gonad weight to somatic tissue weight means that this lag is not an influence on a true GSI. An explanation of how the use of one mantle lobe for egg counts in females was adjusted for in the analysis should also be included.

Reporting

GSI is typically expressed as the ratio of gonad tissue weight to body weight. In mussels, the gonad material is contained mainly within the mantle lobes and for this reason mantle weight may be used as a proxy for gonad weight. The text indicates that dry mantle weight and body weights were used in calculating GSI; however, the analysis indicates the GSI expressed as mantle weight. Figure 3.5 shows logged values above 0, so it appears that log10(Mantle Dry Weight) with log10(Length) as a covariate was used for the analysis as opposed to the ratio of mantle weight to body weight. As the data are not shown in the report, it is difficult to determine what has been used in the analysis and this should be clarified in future reports.

Given the uncertainty surrounding the mortalities throughout the study, additional methods of estimating survival should be considered. The terms of reference recommended the determination of survival without specifying "survival time out of water" method as described in Hellou and Law (2003). This immune response is very sensitive and would represent the number of days an animal can remain alive after being taken out of water and held at a uniform

[1] plug weight = uniform shaped subsample of egg mass extracted from the female mussel's egg mantle which is used to estimate the female's total egg abundance.

temperature. The more stressed mussels would be expected to survive for the shortest period time. The request to investigate survival was interpreted as counting the number of mussels alive at the end of the exposure relative to those placed in the socks.

The mussels experienced a 35% mortality during the deployment period. Lack of an identified cause of mortality prevented an analysis of site differences from being conducted. Without this information, it is difficult to draw conclusions about the significant differences in growth found between sites. The timing of mortality during deployment could provide information regarding the length of time density differences had to affect growth. Density dependent effects on growth rate, and thus differential mortality, may have resulted in density differences that affected growth rate. The inclusion of density as a variable should be considered.

Two separate composite mussel samples from 9 sites were used to conduct chemical analysis of metals and organics within mussels. There were significant differences between areas for lead and copper and significant differences between seasons for cadmium, copper, mercury and zinc. The use of more than 1 composite sample per site per time would allow for the examination of variance in samples where seasonal differences exist for selected metals. The use of additional composite samples would help indicate if differences over time were greater than random variation between samples. Taking multiple samples per site would allow the chemical analysis to be completed on the same scale as the rest of the data and would greatly increase the power of the statistical test to show differences.

Figures labeled Least Square Means showing differences between sites are assumed to be the Tukey's test results rather than the raw data. This should be indicated in the figure title.

Figures 3.1 and 3.3 are both labeled as condition factor; however, there is a 100 fold difference in values. If the condition factor in Figures 3.1 and 3.3 is calculated as g/cm^3 using the length, width, height, and whole animal wet weight values from Tables 3.2 and 3.5, then condition factor should range between 0.4 and 0.5. There appears to be a scaling problem between the two figures as the condition factor in Figure 3.1 ranges between 4 and 5 while, in Figure 3.3, it ranges between 0.04 and 0.05. The is no presentation of results for a multiple comparisons test on the November 2008 data, but the test refers to statistically significant differences between sites. This comparison should be presented.

While discussing the results of the egg counts of mussels in 2009, the author states that the females from site 5 have fewer eggs (Page 11), but it is not stated if the difference is statistically significant.

Conclusions

In general, the sampling program is well designed and the study report provides good baseline information for future monitoring. However, there are some improvements that could be made to the report. The use of improved statistical analysis methods, rationales for site locations, and information regarding site conditions, including currents, effluent discharge locations, and circulation within the harbour would improve the quality of the report. Integration of this information with other contextual and monitoring information is critical as reasons for the patterns observed can only be determined in context. The results of this study need to be integrated with the results of other baseline monitoring programs and an overall preconstruction status of the area determined.

References

Hellou, J., and R.J. Law. 2003. Stress on Stress Response of Wild Mussels, *Mytilus edulis* and *Mytilus trossulus,* as an Indicator of Ecosystem Health. Environmental Pollution. 126(3): 407-416).

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Date: 9 March 2010

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ISSN 1919-3750 (Print)
ISSN 1919-3769 (Online)
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La version française est disponible à l'adresse ci-dessus.

CORRECT CITATION FOR THIS PUBLICATION:

DFO. 2010. Science Review of a Preconstruction Caged Bivalve Study for the Marine Baseline Monitoring Program at Sydney Harbour, Nova Scotia. DFO Can. Sci. Advis. Sec. Sci. Resp. 2010/007.